Cryopreservation of leishmania

1. Distribute the cells grown in stationary phase for 10^8 cells per vial.
2. During harvesting the cells at 3500 g, 4 ^oC for 5 min, label the sterile cryovials with permanent marker giving the strain name and the date/year.
3. Remove the supernatant.
4. Suspend the cells in sterile, 10% glycerol (or DMSO) in M199 supplemented with 10% HIFBS at 0.5~1ml per vial.
5. Keep in ice for 30 min.
6. Transfer the samples to -20 ^oC overnight.
7. Transfer the samples to -80 ^oC overnight.
8. Put the samples into a tank with liquid phase of nitrogen.
9. Make a note of where they are stored and record in the notebook.

※ Transferring the cells from 4 ^oC to -20 ^oC and subsequent transfer to -80 ^oC and liquid nitrogen tank should be done as quickly as possible.

※ Do not store the cells at -20^oC and -80 ^oC for too long, the viability of the cells will decrease substantially.

Thaw out of cryopreserved leishmania

1. Take one cryovial out of the liquid nitrogen tank.
2. Let the cryovial warm to remain a little ice, quickly transfer the vial to the laminar flow hood.
3. Transfer the cells with sterile transfer pipette to 50 ml sterile centrifuge tube.
4. Slowly add the fresh medium drop by drop up to 5ml. After adding each drop, gently mixing the tube by hand. The initial drop is important for optimal cell viability. Later, the number of drops can be increased after each shaking.
5. Centrifuge the cells at 3500g, 4 ^oC for 5 min.
6. Remove the supernatant which contains DMSO or glycerol.
7. Suspend the pellet with 4ml/flask fresh medium in 25 cm^2 TC flask.
8. Observe the motility/viability and division of leishmania daily, first, it may grow slowly at the lag phase and proceed grow at log phase and stationary phase.

※ Do not throw away the flasks if there is no growth in the initial few days. One cell is enough for the culture to grow and it may take quite a few days.